PRESENCE OF THE RED EFT WATER-DRIVE FACTOR PROLACTIN IN THE PITUITARIES OF TELEOSTS

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The lactogenic hormone (prolactin), on account of the complexity and diversity of its functions, continues to excite speculation concerning its relation to changing target organs during the course of vertebrate evolution. The role of this hormone in the promotion of lactation in mammals, its effect on the secretory activity of the crop gland in pigeons, and its association with certain aspects of parental care in both mammals and birds are well known. In addition, prolactin is thought to act as a hyperglycemic agent in some higher vertebrates and may be important in regulating various events in the reproductive cycle, such as ovulation and progesterone secretion. Recently, Juhn and Harris (1958) have shown that the lactogenic hormone may participate in the production of new plumage in birds through stimulation of the feather papilla.

The problem of prolactin in the lower vertebrates has been reviewed by Atz (in Pickford and Atz, 1957) with special reference to fishes. There is considerable evidence that this hormone plays a physiological role in the poikilotherms, although direct evidence for its presence is scanty. Blair (1946) found that prolactin (of uncertain purity) stimulated the production of new melanophores in toads, while Pickford and Kosto (1957) have shown that highly purified intermedin-free prolactin promotes melanin synthesis (but not new pigment cell formation) in the partially depigmented melanophores of hypophysectomized killifish, *Fundulus heteroclitus*. The synergic action of prolactin on the melanocyte-stimulating effect of intermedin (new pigment cell formation) was also demonstrated. The acceleration of molting in prolactin-treated newts, *Diemyctylus* (= Triturus) *viridescens,* observed by Chadwick and Jackson (1948), may have resulted from stimulation of the endogenous release of thyrotropin rather than to a specific action of prolactin since the recipients were not hypophysectomized. The lactogenic hormone stimulates the secretion of jelly by the oviducts of anurans (de Allende, 1939; Houssay, 1947 and later papers cited by Pickford and Atz, 1957; de Allende and Orias, 1955). Positive results could be obtained even after castration or hypophysectomy and the gonadotropins were ineffective. The gonadotropic effects of prolactin on the European newts, *Triton cristatus* and *T. alpestris,* and the stimulation of sperm release in male dogfish, *Scyliorhinus caniculus,* reported by Tuchmann-Duplessis (1948, 1949) and Carlisle (1954), respectively, are interesting but require further investigation.

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A most curious and interesting reaction to prolactin was described by Chadwick (1940) in the immature, terrestrial (red eft) stage of *Diemyctylus viridescens*. The red efts migrated to water within a few days after injection of the hormone and developed the olive pigmentation and strongly keeled tail of the aquatic adult. Similar results were reported by Tuchmann-Duplessis (1949) in experiments with immature terrestrial stages of *Triturus alpestris*. In a further study of this problem, Grant and Grant (1956, 1958), using hypophysectomized efts to exclude the reflex release of other pituitary factors, have confirmed that prolactin is directly responsible for initiating the change in habitat preference. Hypophysectomized efts receiving intraperitoneal injections varying from 8 to 0.04 mg. of highly purified prolactin (C. H. Li) migrated to the water compartment of the vivarium but failed to develop the pigment and morphological characteristics of the complete "water-drive syndrome." The total response observed by Chadwick must therefore involve other hormonal factors. In developing a more decisive test for the water hormone, Grant (1958 and unpublished data) has estimated that a minimum dose of 1.4 μg. of prolactin is necessary to elicit the response in 50% of the hypophysectomized efts tested.

Although the indirect evidence cited above is strongly in favor of the hypothesis that prolactin plays a natural role in the physiology of the lower vertebrates, direct evidence for the presence of this hormone in the poikilotherm pituitary is limited and requires confirmation. Leblond and Noble (1937), using the pigeon crop test, obtained weakly positive results of an indecisive nature with pituitary implants of turtle (*Kinosternon odoratum*), frog (*Rana pipiens*) and some species of teleostean fishes (*Ameiurus nebulosus* and *Lepomis gibbosus*) although others were negative. Foglia (1940) found that pituitaries of the toad, *Bufo arenarum*, stimulated the crop gland of the pigeon. Carlisle (in Medawar, 1953) reports that dogfish pituitary contains a factor which promotes lactation in the mammary gland, but the presence of oxytocin may be suspected. More recently Lehrman (in Pickford and Atz, 1957) has demonstrated prolactin-like activity in pollack pituitary brei (*Pollachius virens*) by means of the pigeon crop test. However, negative results were obtained with a lyophilized powder derived from hake, pollack and cod (Wilhelmi, Lot F80x) (Lehrman, personal communication). The data of Fonseca Ribiero and Tabarelli Neto (1943), with alcohol-preserved pituitaries of the teleost *Prochilodus harti*, depend on the validity of the anuran oviduct test. Chadwick (1941), using the red eft test, demonstrated the presence of a "water-drive factor" in the pituitaries of the toad, *Bufo americanus*, the water snake, *Natrix* sp. and the chicken. The pituitaries of two salamanders (*Plethodon metcalfi* and *Desmognathus fuscus*) and of two snakes (*Diadophis* sp. and *Thamnophis* sp.) gave negative results.

The present investigation was undertaken to obtain further information on the distribution of prolactin among the lower vertebrates. Pituitary glands of pollack, *Pollachius virens*, carp, *Cyprinus carpio*, and killifish, *Fundulus heteroclitus*, were tested for the presence of the water-drive factor by means of the red eft test.

Materials and Methods

The pituitary material was collected and prepared by one of us (G. E. P.). The wet weight of an average gland varies with the species, the size of the fish, the stage of the reproductive cycle, and possibly also the sex. In any given sample, fishes of both sexes and different size ranges were taken together so that the approximate number of glands injected per eft is less meaningful than the actual weight of the material (Table I). In the case of *Fundulus* it should be noted, however, that unpublished data of B. Kosto have shown that the mean weight of the pituitary expressed in terms of body weight is less in winter (sexual regression) than in spring. The mean index, Pituitary Wt./Fish Wt. in mg. per cent was 0.36 in September and October, 0.51 in April. (Both sexes combined, no significant sex differences were noted.)

Pollack (Lots 1, 2, 3, 4): Glands were collected from fish in the pre-spawning condition at Wilson's Beach, Campobello Island, on June 28, 1954. Fish were brought in in the morning and the glands removed within two hours, wrapped in

TABLE I

Doses and calculated numbers of pituitaries involved for each lot of teleost material tested

Lot numbers	Number of test animals each lot	Total dose in mg.	Approx. number of donor pituitaries per dose
Pollack: No. 1	4	10.0	0.8
	2	20.0	1.8
No. 2, 4*	4	10.0	1.1
	2	20.0	2.2
No. 3	4	10.0	1.2
	2	20.0	2.4
Carp: No. 5, 6, 7*	4	20.0	0.8
	2	40.0	1.6
Fundulus: No. 9	4	19.9	70.0
	2	39.8	140.0
No. 10	4	19.5	28.0
	2	39.0	56.0
No. 11	4	19.7	20.0
	2	39.4	40.0
No. 12	2	19.8	24.6

* Each sample tested with same numbers of efts.

"Parafilm" in lots of 5 glands each, and frozen immediately. After four years storage in a closed can in the deep freezer, it was found from their appearance that many of the glands were partially desiccated. The average weight was approximately 50% of the expected wet weight (ca. 20 mg.). Therefore, in making up dilutions for comparison with carp and *Fundulus* pituitaries (not semi-desiccated) it was assumed that 100 mg. $\equiv$ 200 mg. wet weight. Pollack glands were weighed in lots of ca. 100 mg. and homogenized in 2 ml. of 0.6% NaCl. The brei was kept frozen until used. *Carp* (Lots 5, 6, 7): The glands were taken from fish collected in the Connecticut River on October 22, 1957. Although late in the season, some of the males had flowing sperm and many of the females had well-developed ovaries. After eight months storage in closed vials in the deep freezer the glands were weighed in lots of 200 mg. each (9–12 glands) and homogenized, as in the case of the pollack material, in 2 ml. of 0.6% NaCl.

Fundulus (Lot 9): A total of 1700 glands weighing 199.5 mg. was prepared from freshly captured fish collected near New Haven, Connecticut, in October and November, 1957, plus a few from aquarium-kept fish of the same batch killed in December, 1957. All were in a state of complete sexual regression. The material was frozen in closed vials to prevent desiccation. The brei was prepared seven months later; homogenization was difficult, probably on account of the tough neural processes and diminished glandular material at this season of the year, but a smooth homogenate was finally prepared and diluted to 2 ml. in 0.6% NaCl.

(Lot 10): The greater part of the material (280 glands) was taken from pre-spawning fish captured near New Haven, Connecticut, *ca.* May 1, 1955 and kept up to ten days in the laboratory; 80 glands from freshly captured, early spawning fish caught June 7–8, 1955, were added to bring the total to the required weight. The material was stored for two years in closed vials in the deep freezer and the brei was prepared in the usual manner from a total of 360 glands weighing 195.5 mg.

(Lot 11): Two hundred glands weighing 197.5 mg. taken from freshly captured fish at the beginning of the spawning season, June 7–8, 1955, were prepared as described above.

(Lot 12): Three hundred and twenty glands weighing 129.0 mg. taken from freshly captured fish during the period of sexual regression in September and October, 1955 and kept frozen for two and one-half years in closed vials were homogenized in 1.3 ml. of 0.6% NaCl so that the concentration was equivalent to that used in the preceding samples.

In addition to the above material, tests were conducted on a lyophilized preparation (Wilhelmi, Lot F80x) derived from a mixture of hake, pollack and cod. The bulk of the glands were taken from hake (*Urophycis tenuis*) in post-spawning condition. The material was collected at Wilson's Beach, Campobello Island, New Brunswick, in the summer of 1952.

A number of efts collected near Petersham, Massachusetts in August and September, 1958 were hypophysectomized and kept for a period of two weeks before treatment. The animals varied in weight from 0.63 to 1.48 gm. and all were considered to be well removed from the naturally occurring water-drive phase of their life cycle. Each animal received intraperitoneal injections on each of two separate days of fish pituitary brei delivered at a standard volume of either 0.1 ml. or 0.2 ml. per injection. After the first injection the efts were placed in containers with a land and a water area and the time of their migration to water was noted. Changes in weight and length during a period of four weeks after the first injection were recorded in most instances. The data for the doses administered and the calculated number of pituitaries involved are given in Table I.

Results

The results of the above experiments are summarized in Table II.

(a) Tests with pollack: Most animals tested with pollack pituitary failed to show the water-drive response. In all respects they appeared as non-treated hypophysectomized efts, their skin dark and dry as layers of cornified epithelium built up in the absence of normal molting factors presumably involving TSH. Two animals receiving brei from Lot 1 gave a partial response. Their skin sloughed off in rough patches and the efts entered water at irregular intervals, remaining there for several hours at a time. One animal, however, treated with Lot 4 material, gave a positive reaction by assuming the water habitat for a period of a week, during which time all of the old cornified epithelium sloughed off revealing the smooth, non-granular skin characteristic of the aquatic phase. Changes in weight and length were erratic but some individuals (8) undoubtedly grew slightly.

(b) Tests with carp: Fourteen out of the 18 individuals treated with carp pituitary gave a positive response on an average of about ten days following the

Table II

Results of water drive studies following treatment of efts with various teleost pituitary preparations

Donor material and lot numbers	No. test animals*	Wt. change per cent 4 weeks	Length change per cent 4 weeks	Results		Days to water average
				Positive	Negative	
Fundulus (post-spawning)						
Lot No. 9	6	not recorded		5 (1 dead)	0	9.2
Lot No. 12	2			2	0	8.0
Fundulus (chiefly pre-spawning)						
Lot No. 10	6	+7.41	+6.35	6	0	7.6
Fundulus (early spawning)						
Lot No. 11	6	not recorded		0	5	
Pollack (pre-spawning)						
Lot No. 1	6	loss and gain		2 (partial)	4	
Lot No. 2	6	loss and gain		0	6	
Lot No. 3	6	loss and gain		0	6	
Lot No. 4	6	loss and gain		1	5	8.0
Carp (late spawning)						
Lot No. 5	6	+8.41	+4.46	4	2†	12.0
Lot No. 6	6			5 (1 dead)		7.2
Lot No. 7	6	+8.70	+3.20	5 (1 dead)		12.6
F80x Wilhelmi—lyophilized hake, pollack, cod	6	not recorded		0	6	
Controls	16	−5.77	none	0	16	

* See Table I.
† See text.

initial injection and remained in water for periods of from two to three weeks. The four unresponsive individuals may be discounted. Two receiving Lot 5 brei failed to give even a partial response, but one of these was poorly injected so that some of the preparation was lost, while the second individual was suffering from an acute fungus infection. An additional two animals died shortly after the second injection, before any response could have been expected. The efts showed a mean increase in weight and length. All animals molted normally.

(c) Tests with *Fundulus:* Injection of pituitary brei from fish in sexual regression (Lots 9 and 12) yield positive results in all animals tested, with the exception of one which died shortly after the first injection. The animals went to water between eight and nine days after their first treatment and molted normally. It is interesting to note that Lot 10, largely prepared from laboratory-kept animals in the early pre-spawning condition, also gave positive results, while tests with Lot 11 brei from spawning animals were entirely negative. Efts receiving Lot 10 increased significantly in weight and length; the other preparations were not studied in respect to the growth response. Animals receiving Lot 11 molted regularly, indicating that the preparation contained at least some endocrine activity.

Injections of the lyophilized preparation F80x gave negative results in all animals tested.

Controls remained in the terrestrial phase, did not molt, lost weight and showed no change in length.

Conclusions

These tests present convincing evidence that a prolactin-like substance is a naturally occurring factor in the pituitary glands of teleost fishes. Carp brei (Lots 5, 6, 7) gave a positive response as did material prepared from *Fundulus* (Lots 9, 10, 12) in the pre- and post-spawning conditions. The nature of this response indicates that this material, administered in doses of 10 or 20 mg., contained lactogenic hormone well above the estimated threshold of 1.4 μg. for initiation of the water-drive response. That there was no apparent difference in the nature and time of response between animals receiving 10 and 20 mg. of brei agrees with Grant (unpublished data) who has shown that at or above the 3 μg. level total response can be expected in all efts treated, provided they are in a healthy condition.

One of the animals receiving pollack preparations showed positive water-drive, while two others gave a partial reaction. Although the response was very weak in this case, the presence of prolactin in small amounts is indicated. This is in agreement with the work of Lehrman (in Pickford and Atz, 1957) who obtained positive results with pollack brei on the pigeon crop test. Both the present series of tests and those of Lehrman's gave negative results with the lyophilized powder F80x.

The fact that pituitaries taken from animals near the end of the spawning season and from those in the post-spawning condition, gave the strongest response is interesting. Lot 11 brei from early spawning killifish gave negative results while pollack brei from pre-spawning animals produced a very weak response. In the latter case the possibility of deterioration of the sample, which was partially dehydrated after four years' storage in the deep freezer, cannot be excluded, but the glands taken from *Fundulus* in June, 1958 are believed to have been in perfect condition. Further experiments are needed to exclude a possible unexplained deterioration of the sample. These tests may indicate a seasonal depletion in prolactin associated with the reproductive cycle, but the results are not decisive. Negative results with the lyophilized powder, chiefly from post-spawning hake, conflict with this hypothesis.

Summary

It has been demonstrated, by means of the red eft water-drive test, that a prolactin-like hormone is present in the hypophysis of teleostean fishes. Pituitary

extracts from late spawning carp (*Cyprinus carpio*) and pre- or post-spawning killifish (*Fundulus heteroclitus*) gave a positive response in all instances. Pollack pituitary brei (*Pollachius virens*) from pre-spawning fishes gave only a weak response, and wholly negative results were obtained with an extract of *Fundulus* pituitary glands from fish taken at the beginning of the spawning season. While these data suggest a possible period of depletion during the early spawning phase of the sexual cycle, the findings require confirmation. The experiments also demonstrated that the pituitary of the three species investigated contains a growth-promoting factor and a molting hormone, presumably somatotropin and thyrotropin, respectively.

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